

EXHIBIT D

Rats, anesthetized with ketamine and barbiturate, were subjected endotracheal intubation and mechanical ventilation. Halothane was administered for continued anesthesia. A thoracotomy was then performed. A silk thread was then passed around the left anterior descending coronary artery (LAD). The LAD was occluded for 30 minutes and then reopened. The chest was then closed and the animal allowed to recover.

Peptides were initially dissolved in DMSO, then diluted in saline so that the final volume was 5 mg of peptide was contained in 3 ml of saline. The indicated peptide was administered by constant infusion beginning 20 minutes after the onset of the occlusion and continuing for 43 minutes. The occlusion was released 10 minutes after the start of the infusion. Control animals received the diluent DMSO in 3 ml of saline at a perfusion rate of 70 μ l/min.

Twenty-four hours after treatment, the animal was anesthetized and sacrificed. The heart was cut into 4 horizontal sections (i) 25% from the base; (ii) 50% from base; (iii) 75% from base; and (iv) just below the site of ligation. The slices were cut on a microtome and stained for TUNEL, an indicator of apoptosis. The percentage of apoptotic nuclei found on microscopic examination is shown in Table 1, below.

Table 1. Effect of VDV peptides on the percentage of apoptotic nuclei by microscopic examination following LAD occlusion.

	Control	Peptide (5 mg)		
		SVDVEY	TVDVEY	YVDVEY
Percentage of apoptotic nuclei (\pmSEM)	16.0 \pm 2.7 N=10	8.1 \pm 0.0 N=2	8.0 \pm 2.3 N=11	11.0 \pm 3.0 N=5
Reduction in apoptosis		50.4%	50.0%	31.3%

METHOD

Five sites were arbitrarily (randomly) selected from each quadrant. The total number of nuclei per high-powered field was counted. The number of TUNEL positive nuclei was counted, and the percentage of TUNEL positive nuclei per high-powered field recorded. The average of the five sites is the number of TUNEL positive cells per quadrant. The average for each section was calculated. Thus each heart has four slices and four quadrants per slice are examined. Each quadrant represents the mean of five measurements. All studies were done by an observer blinded to the origin of the samples.